

Appl. No. 09/837,711  
Amdt. dated October 12, 2005  
Amendment under 37 CFR 1.116 Expedited Procedure  
Examining Group 1652

PATENT

**Amendments to the Claims:**

This listing of claims will replace all prior versions, and listings, of claims in the application:

**Listing of Claims:**

1-70. (Cancelled)

71.(Currently Amended) A method for synthesizing a glycoside product,

(a) selecting a glycoside product which is a substrate for a wild-type glycosidase enzyme having two catalytically active amino acids with carboxylic acid side chains within the active site;

(b) combining in a reaction mixture

(i) a mutant form of the glycosidase enzyme, said mutant enzyme having one of said catalytically active amino acids of the glycosidase enzyme substituted with a different amino acid of comparable or smaller size, said different amino acid having a non-carboxylic acid side chain, wherein said mutant glycosidase does not catalyze the hydrolysis of the oligosaccharide glycoside product and retains the activity to synthesize the oligosaccharide glycoside product; and

(ii) a glycosyl donor molecule comprising a ~~substituent that is a good~~ sugar linked to a leaving group and a glycoside acceptor molecule ~~corresponding to the glycosidase enzyme and to the glycoside product to be synthesized~~, said glycosyl donor molecule having a  $\beta$  configuration and said glycoside acceptor molecule having an  $\alpha$  configuration, or vice versa, wherein said glycoside acceptor molecule and said glycosyl donor molecule are selected so as to form a glycosidic bond which is hydrolyzed by the wild-type glycosidase enzyme; and

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(c) allowing the mutant glycosidase enzyme to enzymatically couple the glycosyl donor molecule and glycoside acceptor molecule to synthesize the glycosyl glycoside product.

72. (Currently Amended) The method of claim 71, wherein the wild-type glycosidase enzyme is a stereochemistry retaining enzyme in which the carboxylic acid side chain of one of said catalytically active amino acids in the wild-type glycosidase enzyme active site functions as an acid/base catalyst and the carboxylic acid side chain of the other catalytically active amino acid functions as a nucleophile, and wherein the amino acid having the nucleophile carboxylic acid side chain is replaced in the mutant enzyme.

73. (Currently Amended) The method of claim 72, wherein the wild-type glycosidase enzyme is a  $\beta$ -glycosidase.

74. (Previously Presented) The method of claim 73, wherein the glycosyl donor molecule is  $\alpha$ -glycosyl fluoride.

75. (Previously Presented) The method of claim 73, wherein the  $\alpha$ -glycosyl fluoride is  $\alpha$ -glucosyl fluoride.

76. (Previously Presented) The method of claim 73, wherein the  $\alpha$ -glycosyl fluoride is a  $\alpha$ -galactosyl fluoride.

77. (Currently Amended) The method of claim 71, wherein the wild-type glycosidase enzyme is a  $\beta$ -glycosidase.

78. (Previously Presented) The method of claim 71, wherein the enzyme is a  $\beta$ -glucosidase.

79. (Previously Presented) The method of claim 71, wherein the acceptor molecule is an aryl-glycoside.

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80. (Previously Presented) The method of claim 79, wherein the acceptor molecule is a nitrophenyl-glycoside.

81. (Currently Amended) The method of claim 71, wherein the wild-type glycosidase enzyme is a stereochemistry inverting enzyme in which the carboxylic acid side chains of one of said catalytically active amino acids in the active site of the wild-type glycosidase enzyme functions as an acid catalyst and the other carboxylic acid side chain of the other catalytically active amino acid functions as a base catalyst, and wherein the amino acid having the carboxylic acid side chain which functions as a base catalyst is replaced in the mutant enzyme.

82. (Currently Amended) The method of claim 71, wherein the wild-type glycosidase enzyme is selected from the group consisting of  $\beta$ -glucosidases,  $\beta$ -galactosidases,  $\beta$ -mannosidases,  $\beta$ -N-acetyl glucosaminidases,  $\beta$ -N acetyl galactosaminidases,  $\beta$ -xylosidases,  $\beta$ -fucosidases, cellulases, xylanases, galactanases, mannanases, hemicellulases, amylases, glucoamylases,  $\alpha$ -glucosidases,  $\alpha$ -galactosidases,  $\alpha$ -mannosidases,  $\alpha$ -N-acetyl glucosaminidases,  $\alpha$ -N acetyl galactosaminidases,  $\alpha$ -xylosidases,  $\alpha$ -fucosidases, and neuraminidases/sialidases.

83. (Previously Presented) The method of claim 71, wherein the glycoside acceptor molecule is selected from the group consisting of a monosaccharide, an oligosaccharide, and a sugar containing molecule.